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Synthesis of [9,10-^{3}H]-3-azidosalicyl-N-(n-octadecyl)amide

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Summary

The synthesis of [9,10-^{3}H]-3-azidosalicyl-N-(n-octadecyl)amide, which can act as the molecular probe, was consisted of three step reaction. First 3-nitrosalicylic acid reacted with oleyleamine to form 3-nitrosalicyl-N-(9'-octadeceny)aminde, that reduced by tritium to the [9,10-^{3}H]-3-amionsalicyl-N-(n-octadecyl)amide. It then reacted with sodium nitrite and. sodium azide to synthesize [9,10-^{3}H]-3-azidosalicyl-N-(n-octadecyl)amide. Its specific activity and radiochemical purity were 46 Ci/mmol and >98% respectively.

Key words: Synthesis; inhibitor; [9,10-^{3}H]-3-Azidosalicyl-N-(n-octadecyl)amide; Q-binding protein

Introduction

The ubiquinone(Q)-binding protein hypothesis was suggested first by King (1) and Yu in 1978. It is believed that there exist Q-binding protein in NADH-Quinone reductase, succinate-quinone reductase and ubiquinone-cytochrome c reductase (2). QPs, the Q-binding protein in succinate-ubiquinone reductase (SQR), was first isolated from cytochrome b-c_1 particle by Yu and King in 1977 and which could be reconstituted with succinate dehydrogenase into SQR (3), and henceforth the Q-binding protein hypothesis began to be accepted extensively. Since 1985, Yu began to identyfy the Q-binding protein in ubiquinol-cytochrome c reductase (QPc) with tritium-labelled azido-ubiquinone derivatives and a few of Q-binding proteins have been isolated (4). But the Q-binding proteins in NADH-ubiquinone reductase remain to be identified until now. To study the Q-binding hypothesis, we synthesized a series of inhibitors of ubiquinone reactions. We discovered that 3-nitrosalicyl-N-alkyl-amide inhibitors with long side alkyl chain specifically inhibit the ubiquinone reactions and they have inhibitory activity to all the three complexes, which catalyze the quinone reaction (5-8). The property that 3-nitrosalicyl-N-alkylamides specifically inhibit quinone reaction provides us a useful probe to study the Q-binding proteins. If the nitro group is replaced by azido group to be photoaffinity compound (9), it would be possible that tritium-labelled 3-azidosalicyl-N-alkylmide used as a photoaffinity probe for the inhibitory site of quinone reaction and which will provide us much evidence of Q-binding proteins further. Therefore we should synthesize the [9,10-^{3}H]-3-azidosalicyl-N-(n-octadecyl)amide for this study.

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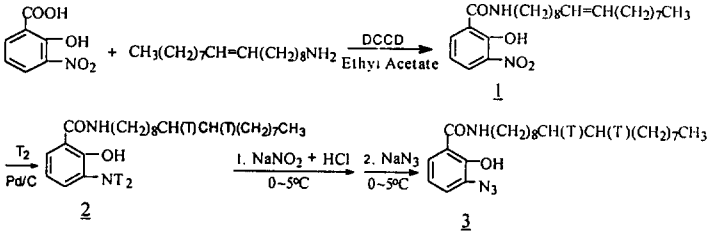
Results and Discussion

Based on the principle of peptide synthesis (10), the building of peptide bond is the acylation of the amino group of an amino acid by the carboxyl group of a second amino acid. The carboxyl group of 3-nitrosalicylic acid with the amino group of oleyleamine condensed to the 3-nitrosalicyl-N-(9'-octadeceny)amide under presence of N,N'-dicyclohexylcarbodiimide (DCCD) in the ethyl acetate solvent system. This compound was used to prepare the [9,10-^{3}H]-3-aminosalicyl-N-(n-octadecyl)amide required to further synthesis of the desired compound, [9,10-^{3}H]-3-azidosalicyl-N-(n-octadecyl)amide. The synthesis route of [9,10-^{3}H]-3-azidosalicyl-N-(n-octadecyl)amide was shown as follows:



When the compound **1** was synthesized with the methods of Wilson et al (11-13), we found that the yield was higher if ethyl acetate instead of tetrahydrofuran (THF) acted as a solvent. Moreover, some of dicyclohexylurea that produced in the reaction, would dissolve in the solvent and mix up with product **1** which made it difficult to purify 3-Nitrosalicyl-N-(9'-octadeceny)amide. We also found the solubility of dicyclohexylurea is less in ethyl acetate than in THF. So ethyl acetate is better solvent than THF to this reaction. To avoid that, we used as little as solvent for less being mixed with product**1**.

In the preparation of compound **2**, if the nitro group of 3-nitrosalicylic acid was reduced to the amino group before synthesis of the compound **1**, it would be too unstable to synthesize the compound **1**. So tritium gas was used to reduce the double bounds of compound **1** and its nitro group to the amino group. For getting much higher specific activity of compound **2**, carrier-free tritium gas should be used. Moreover, in order to make this reaction complete, the reaction time should be longer. Since the compound **2** is unstable in the air, the separation of product from the reaction mixture by filtration should be performed in nitrogen atmosphere. When [9,10-^{3}H]-3-aminosalicyl-N-(n-octadecyl)amide was obtained, it should be immediately dissolved in absolute ethanol for using in the next step reaction.

In order to raise the yield of compound **3**, excess of sodium nitrite and sodium azide must be added. The reaction must be in the dark and at the ice bath to maintain the temperature between 0-5°C. Otherwise the yield of compound **3** was very low and it was very difficult to purify the compound. When the compound **3** was obtained, its UV spectrum was recorded in UV spectrometer (see Figure 1). The spectrum of compound **3** was the same as the spectrum of standard sample (see Figure 2). It was considered that the compound **3** was the desired compound. When it stood in the air for few hours, it would be changed into pink-red colour. So it must be stored in the nitrogen.

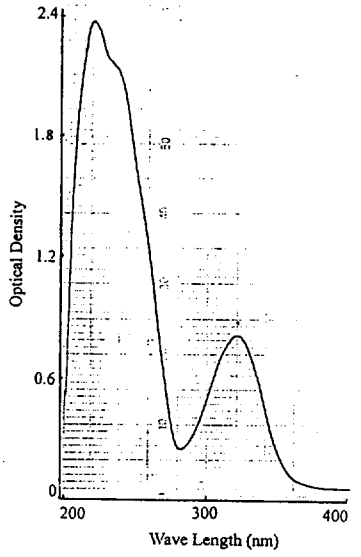


Figure 1 Spectrum of compound 3

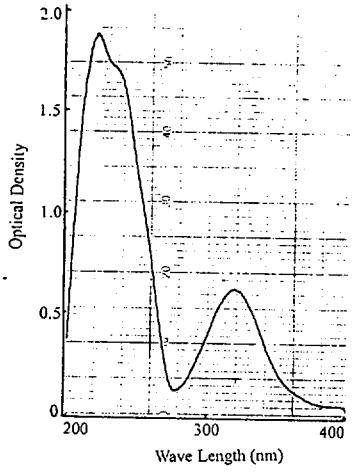


Figure 2 Spectrum of standard sample

Experimental

Oleyleamine and N, N'-dicyclohexylcarbodiimide were obtained from Fluka. Sodium azide was from Merck. 10% Palladium on activated carbon was prepared by ourselves. 3-Nitrosalicylic acid was from Beijing Chemical Co. All other chemicals were obtained commercially at the highest purity available. Ultraviolet spectra were obtained on the UV-210 spectrometer using ethanol as solvent. Radiochemical purity was determined in a thin layer radioscanner Model RTLS-A. Tritium was counted using a Packard Liquid Scintillation Counter, Model FJ-353G. Elemental analysis was determined with Elementary Analyzer Model 1106. MS spectra was recorded on HP-5988 GC-MS spectrometer.

(1) Synthesis of 3-Nitrosalicyl-N-(9'-octadecenyl)amide 1

To 1.83g (10 mmol) of 3-nitrosalicylic acid dissolved in 15 ml of ethyl acetate was added 2.67 g (10 mmol) of oleyleamine dissolved in 30 ml of ethyl acetate. 4 g (20 mmol) of N, N'-dicyclohexyl-carbodiimide dissolved in 5 ml of ethyl acetate was added to this solution. The mixtures then stirred at room temperature for 6 hours. After the reaction was completed, 0.5 ml of glacial acetic acid was added to decompose excess of N, N'-dicyclohexylcarbodiimide. When the solvent was filtrated and removed, 3-nitrosalicyl-N-(9'-octadeceny)amide was thus obtained. The product 1 was purified by recrystallization from ethanol/water to give 2.29 g of yellow crystals, m.p. 76-77°C, yield 53%. m/z: 432(M^+) . Analysis, calculated for $C_{25}H_{40}N_2O_4$, C,69.44; H,9.26; N,6.48. Found, C, 69.28; H, 9.31; N, 6.45.

(2) Synthesis of [9,10-^{3}H]-3-amionsalicyl-N-(n-octadecyl)amide 2

To a 20 ml reaction flask, 10 mg (0.023 mmol) of compound 1, which was dissolved in 2 ml of acetone, and 10 mg of 10% palladium on activated carbon was added. After the reaction flask was cooled by liquid nitrogen and evacuated to 1×10^{-3} mm Hg, tritium was introduced to the reaction flask and maintained at 660 mm Hg before the sample thawed. The mixture of compound 1 and tritium gas was

stirred for 48 hours over the catalyst at room temperature. The reaction flask was then cooled with liquid nitrogen, unreacted tritium was recovered to the tritium storage tank. The catalyst was removed from the product by filtration and to remove labile tritium by the repeated addition of ethanol followed by evaporation. The product 2 was dissolved in absolute ethanol and did not need be purified for using in the following reaction.

(3) Synthesis of [9,10-^{3}H]-3-azidosalicyl-N-(n-octadecyl)amide 3

When compound 2 was obtained, it was immediately dissolved in absolute ethanol and cooled to 0-5°C, 0.1 ml of 36% hydrochloric acid was added and then 0.004 g (0.047 mmol) of sodium nitrite dissolved in a minimal amount of water was added with stirring. It was continual stirred for 40 min. at 0-5°C. After the formation of 3-diazosalicyl-N-(n-octadecyl)amide was complete (judged by using silica gel thin-layer chromatography with a chloroform solvent system), 3 mg(0.046 mmol) of sodium azide dissolved in a minimal amount of water was added to the reaction mixture. The reaction mixture was stirred for further 3 hours in the dark and at the ice bath maintaining temperature between 0-5℃. The mixture was filtrated and the filtrate was poured into 5 ml of water. The precipitate was collected by filtration and recrystallized from ethanol/water. The compound 3 was dissolved in ethanol, which was used to determine its chemical quantity (yield was 90%) by UV spectrometer and count its radioactivity with liquid scintillation counter. It then spotted on Whatman No. 1 paper to perform chromatography for determining its radiochemical purity on thin layer radioscanner. Its radiochemical purity and specific activity were >98% and 46 Ci/mmol respectively.

Acknowledgements

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